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MARINE TOXICITY TEST SUMMARY
14 Toxicity Tests

OSEI Corporation, in its attempt to prove ***"Oil Spill Eater II"*** is virtually non-toxic, had the following tests performed:

The **MYSIDOPSIS BAHIA (or Mysid)** is one of the more sensitive marine organisms found in the oceans. LC50's (Lethal Concentration) is the level in which there is mortality with 50% of the species being tested. The lethal concentration calculated for OSEII on the Mysid was calculated once 10% of the test species showed equilibrium problems or mortality. At 96 hours, only 10% of the test species showed equilibrium problems or mortality at a calculated level of 2100 mg/L or 2,100 parts per million. This shows OSEII to have a low toxicity level, and had a true LC50 been performed the toxicity level would have been even lower.

The **MUMMICHOG (Fundulus Heteroclitus)** a somewhat larger organism (1 to 1.5 inches long) was tested to see how toxic OSEII was to it. 5,258 mg/L was established. 5,285 parts per million shows a very little toxicity for the Mummichog when exposed to Oil Spill Eater II.

MEDIAN LETHAL CONCENTRATIONS (LG50's) were calculated on Artemia Salina. The tests were run for 48 hours. OSEII alone tested greater than 100 mg/L so the true LG50 was not determined, but OSEII toxicity was greater than the EPA's cut-off for approving a product for the National Contingency Plan. There were other interesting facts involved with this toxicity test. The test calculation was based on using our product at a stronger concentration than our instructions allow. So at our instructed use rate, the toxicity level would have been even lower, even though the test was based on 100 mg/L or greater value. No. 2 fuel oil was tested alone and showed a level of 12.6 mg/L at 48 hours and No. 2 fuel oil and OSEII together at 48 hours showed a level of 29.4 helping

prove our point that once OSEII is applied, it immediately starts detoxifying hydrocarbons so bacteria can devour the hydrocarbons. (It is more beneficial to the environment to apply OSEII immediately, than to wait around for evaporation or to try to pick up the hydrocarbons mechanically.)98

OSEI Corporation feels the toxicity tests run in conjunction with OSEII help prove OSEII is virtually non-toxic. The EPA established that 35 mg/L LC50 was acceptable for a particular product to be used on the Exxon Valdez spill. If you compare OSEII to this established toxicity of 35 mg/L, then OSEII is far less toxic than that.
OSEI Corporation had two (2) fresh water toxicity tests run also. Environmental Canada, the U.S. EPA's equivalent in Canada, performed a toxicity test on rainbow trout. Rainbow trout are very sensitive fresh water species. The LC50 was greater than 10,000 mg/L. This shows OSEII to have virtually no toxicity in fresh water as well as salt water.
The other fresh water test was run on fathead minnows for the physical engineer in Plano, Texas, USA. We were attempting to prove that hydrocarbons which have had OSEII applied to them and then washed in the storm drain would not add any toxicity to the storm drain.
Two gallons of gasoline was poured onto a low area in a commercial business parking lot, and OSEII was applied, allowed to set 3 minutes, and then washed to another low area for collection.

Approximately 1 ••• gallons of runoff was collected and taken to the lab where a 48 hour fathead minnow survival test was initiated. The resulting LC50 test was 9,300 mg/L which shows that gasoline which has had OSEII applied to it is rendered virtually non-toxic.
This helped alleviate the physical engineer's concerns for adding anything toxic to the storm drain and ultimately to a creek, river or lake.
This test shows that using OSEII would help reduce the toxicity to storm drains from rain water runoff. If OSEII is used periodically to clean the parking lot allowing the site to stay within its NPDES permitted discharge levels.

Sincerely,
Steven Pedigo
Chairman

SP/eem99 OIL SPILL EATER INTERNATIONAL, CORP.

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SUMMARY
EPA/NETAC TOXICITY TEST
MYSIDOPSIS BAHIA

The Environmental Protection Agency in Gulf Breeze, Florida tested OIL SPILL EATER II Concentrate, for toxicity using a sensitive species named “Mysidopsis Bahia”. This test was in conjunction with Efficacy Tests performed by the EPA and NETAC.

The LC50 for the acute (96 hr.) test was greater than 1,900 and up to 10,000 mg/L which shows OSE II to be virtually non-toxic.

The EPA allowed the use of Inipol during the Valdez Spill and Inipol’s LC50 was 135 mg/L which would seem to OSEI, Corp to be somewhat toxic considering Environmental Canada’s cut off is 1,000 mg/L.

A second LC50 was performed at 7 days to see if there was any problem with chronic toxicity. The LC50 was 2,500 mg/L, which once again shows OSE II to be virtually non-toxic even when the species was exposed in a closed environment for 7 days. It would be extremely difficult for a species to be exposed to OSE II for 7 days in an open system due to currents, wind and tidal actions.

This 3rd party, U.S. EPA Toxicity Test absolutely proves OSE II is virtually non-toxic.

By: Steven R. Pedigo
Chairman/OSEI, Corp.

SRP/AJL100

OIL SPILL RESPONSE BIOREMEDIATION AGENTS
EVALUATION METHODS VALIDATION TESTING
DISCUSSION OF RESULTS

The following data are provided for the oil spill response bioremediation agent producer as a means to begin to assess how this bioremediation agent may behave in response to an oil spill in the environment.
The Tier II 96-hour toxicity test data was conducted with Mysidopsis bahia test species. Mortality was the single measure response, therefore, survival data were used to calculate the 96-hour LC50. LC50 is the lowest concentration effecting 50% mortality of the test organism during a 96 hour exposure period. Sub-lethal and lethal responses were noted at concentrations between 1,000-10,000 mg/L (> 1,900 mg/L) following acute exposure of M.bahia to your bioremediation product.
Oil Spill Eater II was shown to cause a statistically significant reduction (p = 0.05) in the survival of Mysidopsis when animals were exposed during a chronic estimator test for a 7 day period. In general, 7 day exposure (2,500 mg/L) correlated well with values calculated following the 96 hour exposure (> 1,900 mg/L).NETAC101

TIER II TOXICITY DATA
TABLE 1

ACUTE TOXICITY VALUES FOR 96 HOUR LC_{50} – MYSIDOPSIS BAHIA

LC_{50} = Lethal concentration of product that will cause the death of 50% of the test species population within a defined exposure time.
a = LC50 presented as a range of test concentrations since data were from 96-hour acute range-finding test.
b = LC50 presented as a single, numerical value since data were from a definitive 96-hour acute toxicity test.
ND = Not Determined

TABLE 2

CHRONIC TOXICITY VALUES FOR 7 DAY LC_{50} – ***MYSIDOPSIS BAHIA***

NOEC = No Observable Effect Concentration
LOEC = Lowest Observable Effect Concentration
CI = Confidence Interval
NE = No Effect
Fecundity = Egg Production

As we indicated prior and to better understand the data presented above we are including a copy of the Evaluation Methods Manual. The Statistical Method Summary is found in Section 4, Method #8, page 40, of the manual and is intended to help a scientist understand the basis of the experimental objectives developed for this test.

Max. Test
Concentration
(mg/L)
Confidence
Interval
(95%)
96 hour LC50
(mg/L)
Product
1,000-10,000[a]
>1,900[b]
Oil Spill
Eater II
10,000
ND
7 Day LC50
(mg/L)
(95% CI)
Endpoints
(mg/L)
Effects
Measurement
Product
NOEC LOEC

5,700
NE
1,900
1,900
1,900
633
Survival
Growth
Fecundity
2,500(mg/L)
(2,225-3,313)

Oil Spill
Eater II NETAC102
Static Acute Toxicity of
Oil Spill Eater II, Batch 329,

To the Mysid, Mysidopsis bahia
Study Completed
March 9, 1990
Performing Laboratory
EnviroSystems Division

Resource Analysts, Incorporated
P.O. Box 778
One Lafayette Road
Hampton, New Hampshire 03842

I. SUMMARY

The acute toxicity of Oil Spill Eater II, batch 329 to the mysid, Mysidopsis bahia, is described in this report. The test was conducted for Incorporated for 96 hours during March 5-9, 1990 at the EnviroSystems Division of Resource Analysts, Inc. in Hampton, New Hampshire. It was conducted by Jeanne Magazu, Peter Kowalski, Robert Boeri, and Timothy Ward.

The test was performed under static conditions with five concentrations of test substance and a dilution water control at a mean temperature of 19.5◦C. The dilution water was filtered natural seawater collected from the Atlantic Ocean at Hampton, New Hampshire. Aeration was not required to maintain dissolved oxygen concentrations above an acceptable level. Nominal concentrations of Oil Spill Eater II were: 0 mg/L (control), 1 mg/L, 10 mg/L, 100 mg/L, 1,000 mg/L, and 10,000 mg/L. Nominal concentrations were used for all calculations.

Mysids used in the test were less than 5 days old at the start of the test. They were produced at Resource Analysts, Inc. and acclimated under test conditions for their entire life. All mysids were in good condition at the beginning of the study.

Exposure of mysids to the test substance resulted in a 96 hour LC50 of 2,100 mg/L Oil Spill Eater II, with a 95 percent confidence level of 100 – 10,000 mg/L. The 96 hour no observed effect concentration is estimated to be 100 mg/L.

IV. METHODS AND MATERIALS

TEST SUBSTANCE:

Oil Spill Eater II (EnviroSystems Sample Number 2351E) was delivered to EnviroSystems on March 5, 1990. It was contained in a 500 ml plastic bottle that was labeled with the following information: Oil Spill Eater II, Batch 329. The sample was supplied by Incorporated. Prior to use the test material was stored at room temperature. Nominal concentrations were added to test media on a weight/vol basis and are reported as mg/L.

DILUTION WATER:

Water used for acclimation of test organisms and for all toxicity testing was seawater collected from the Atlantic Ocean at EnviroSystems in Hampton, New Hampshire. Water was adjusted to a salinity of 11-17 ppt (parts per thousand) and stored in 500-gallon polyethylene tanks, where it was aerated.

TEST ORGANISM:

Juvenile mysids employed as test organisms were from a single source and were identified using an approximate taxonomic key. They were produced and acclimated at the Resource Analysts, Inc. facility for their entire life. During acclimation mysids were not treated for disease and they were free of apparent sickness, injuries, and abnormalities at the beginning of the test. Mysids were fed newly hatched Artemia salina nauplii (EnviroSystems lot number

BS01) once or twice daily before the test.

TOXICITY TESTING:

The definitive toxicity test was performed during March 5-9, 1990. It was based on procedures of the U.S. Environmental Protection Agency (1986, 1987). The test was conducted at a target temperature of 20 ± 2◦C with five concentrations of test substance and a dilution water control. A stock solution was prepared by combining 20.0 g of test substance with 2,000 ml of dilution water. The stock solution was added directly to dilution water contained in the test vessels without the use of a solvent. Nominal concentrations of the test material were: 0 mg/L, 10 mg/L, 100 mg/L, 1,000 mg/L, and 10,000 mg/L.

Twenty mysids were randomly distributed among a single replicate of each treatment. The test was performed in 2 liter glass dishes (approximately 25 cm in diameter and 8 cm deep) that contained 1.0 liter of test solution (water depth was approximately 4 cm). Test vessels were randomly arranged in an incubator during the 96 hour test. A 16 hour light and 8 hour dark photoperiod was automatically maintained with cool-white fluorescent lights that provided a light intensity of 40 eEs-1m-2. Aeration was not required to maintain dissolved oxygen concentrations above acceptable levels. Mysids were fed newly hatched Artemia salina nauplii once per day during the test.

The number of surviving organisms and the occurrence of sublethal effects (loss of equilibrium, erratic swimming, loss of reflex, excitability, discoloration, or change in behavior) were determined visually and recorded initially and after 24, 48, 72, and 96 hours. Dead test organisms were removed when first observed. Dissolved oxygen (YSI Model 57 meter; instrument number PRL-3), pH (Beckman model pHI 12 meter; instrument number PRL-4), salinity (Labcomp SCT meter, instrument number PRL-6), and temperature (ASTM mercury thermometer; thermometer number 2211) were measured and recorded daily in each test chamber that contained live animals.

STATISTICAL METHODS:

Results of the toxicity test were interpreted by standard statistical techniques. Computer methods (Stephan, 1983) were used to calculate the 96 hour median lethal concentration (LC50). The no observed effect level is the highest tested concentration at which 90% or more of the exposed organisms were unaffected.

V. RESULTS

No insoluble material was observed in any test vessel during the test. Biological and water quality data generated by the acute toxicity test are presented in Table 1 and Appendix A, respectively. One hundred percent survival occurred in the control exposure.

The dose – response curve for organisms exposed to the test substance for 96 hours is presented in Figure 1. Exposure of mysids to the Oil Spill Eater II, batch 329, resulted in a 96 hour LC50 of 2,100 mg/L, with a 95 percent confidence interval of 100 – 10,000 mg/L. The 96 hour no observed effect concentration is estimated to be 100 mg/L.

Table 1. Survival data from toxicity test

Nominal Concentration (mg/L)	Number Alive 0hr	24hr	48hr	72hr	96hr	Number Affected 0hr	24hr	48hr	72hr	96hr
0 (control)	1	10	10	10	10	10	0	0	0	0
1	1	10	10	9	9	9	0	0	0	0
10	1	10	10	9	9	9	0	0	0	0
100	1	10	10	10	9	9	0	0	0	0
1,000	1	10	9	9	8	8	0	0	0	0
10,000	1	10	0	0	0	0	-	-	-	-

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TOXICITY TEST FOR ARTEMIA SALINA

To gain acceptance on the U.S. EPA's National Contingency Plan List, we were requested to perform an additional Toxicity Test on Artemia Salina using EPA's Standard Dispersant Toxicity Test.

OSE II Concentrate was presented to the laboratory, but the laboratory refers to the product as a Dispersant instead of OSE II throughout the write-up, since it was a Dispersant Toxicity Test. The Test proved that OSE II Concentrate is once again virtually non-toxic. This particular test proved OSE II helps to detoxify oil. The fuel oil had a higher toxicity rate than did the fuel and OSE II, which shows OSE II to immediately starts reducing the toxicity of hydrocarbons once OSE II is applied. The fuel oils toxicity was 12.4 ppm, and the fuel oil and with OSE II applied showed a drop in the fuel oils toxicity to 29.4, over a 100 percent reduction of the toxicity of the fuel oil. This shows real value in utilizing OSE II since the toxicity of the spilled contaminant would be reduced immediately lesoning the impact of a spill to the associated environment and marine species.

OSE II gained acceptance to the EPA's National Contingency Plan once this test was presented to the EPA.

By: Steven R. Pedigo
Chairman, OSEI, Corp.

SRP/AJL111

Standard Dispersant Toxicity Test with the
OSE II, Batch #9820 and Artemia salina

Authors
Timothy J. Ward
Robert L. Boeri
Performing Laboratory
EnviroSystems Division
Resource Analysts, Incorporated
P.O. Box 778
One Lafayette Road
Hampton, New Hampshire 03842
October, 1990

II. TABLE OF CONTENTS

III. Index of Tables

IV. INTRODUCTION

The objective of the study was to determine the acute toxicity of the dispersant – Batch # 9820, No. 2 fuel oil, and a 1:10 mixture of dispersant and oil to *Artemia salina*, a marine invertebrate. The report contains sections that describe the methods and materials employed in the study, and the results of the investigation. The report also contains an appendix that presents the water quality data collected during the tests.

V. METHODS AND MATERIALS

TEST SUBSTANCE:

The dispersant – Batch # 9820 (EnviroSystems Sample Number 2591E) was delivered to EnviroSystems on August 17, 1990. It was contained in two 1,000 ml plastic bottles that were labeled with the following information: "Batch # 9820". The No. 2 fuel oil (EnviroSystems Sample Number 2599E) was delivered to EnviroSystems on August 28, 1990. It was contained in a 1,000 ml plastic bottle that was labeled with the following information: "# 2 fuel oil".

DILUTION WATER:

Water used for hatching and acclimation of test organisms and for all toxicity testing was formulated at EnviroSystems in Hampton, New Hampshire. Water was diluted to a salinity of 20 parts per thousand and stored in polyethylene tanks where it was aerated.

TEST ORGANISM:

Juvenile *Artemia salina* employed as test organisms were from a single source and were identified using an appropriate taxonomic key. *Artemia salina* used in the test were produced from an in-house culture and were 24 hours old at the start of the test. Prior to testing, *Artemia salina* were maintained in 100% dilution water under static conditions. During acclimation *Artemia salina* were not treated for disease and they were free of apparent sickness, injuries, and abnormalities at the beginning of the test. They were not fed before or during the tests.

TOXICITY TESTING:

Screening tests with the test substances were conducted during October 1 to 3, 1990. The definitive toxicity tests were performed with the dispersant, No. 2 fuel oil, a 1:10 mixture of dispersant and oil, and the standard toxicant, dodecyl sodium sulfate during October 3 to 5, 1990, according to procedures of the U.S. EPA (1984). The tests were conducted at a target temperature of 20 ± 1◦C with five concentrations of each test substance and a dilution water control.

The dispersant and oil stock solutions were prepared by combining 550 ml of sea water and 0.55 ml of test substance in a glass blender jar and mixing the solution at 10,000 rpm for 5 seconds. The combined dispersant and oil stock solution was prepared by mixing 550 ml of sea water at 10,000 rpm and adding 0.5 ml of oil and 0.05 ml of dispersant. This combined mixture was then mixed for 5 seconds. Nominal concentrations of each test material were: 0 mg/L (control), 10 mg/L, 25 mg/L, 40 mg/L, 60 mg/L, and 100 mg/L. Media in each test vessel was added at the beginning of the test and not renewed.

Twenty *Artemia salina* were randomly distributed to each of 5 replicates of each treatment. The tests were performed in 250 ml glass Carolina culture dishes that contained 100 ml of test solution (water depth was approximately 2.5 cm). Test vessels were randomly arranged in an incubator during the 48 hour test. A 24 hour light and 0 hour dark photoperiod was maintained below the dishes. Aeration was not required to maintain dissolved oxygen concentrations above acceptable levels. *Artemia salina* were not fed during the tests.

The number of surviving organisms was determined visually and recorded initially and after 24 and 48 hours. Dead test organisms were removed when first observed. Dissolved oxygen (YSI Model 57 meter; instrument number PRL-18), pH (Beckman model pHI 12 meter; instrument number PRL-4), salinity (Refractometer, instrument number PRL-6), and temperature (ASTM mercury thermometer; thermometer number 2211) were measured and recorded at the beginning and end of each test in one test chamber of each concentration.

STATISTICAL METHODS:

Results of the toxicity test were interpreted by standard statistical techniques (Stephen, 1983). The binomial method was used to calculate the median lethal concentration (LC50) values.

VI. RESULTS

All test vessels containing dispersant appeared clear throughout the test and all test vessels containing oil or oil and dispersant had an oil slick on the surface of the test media throughout the test. Biological and water quality data generated by the acute toxicity tests are presented in Table 1 and Appendix A, respectively. Ninety-nine percent survival occurred in the control exposure. The 48 hour LC50 for *Artemia salina* exposed to the reference toxicant dodecyl sodium sulfate is 38.7 mg/L.

The 24 and 48 hour LD50s from the three toxicity tests are presented in Table 2. The 48 hour LC50s for *Artemia salina* exposed to the test substances are: dispersant/OSE II - >100 mg/L, No. fuel oil – 12.6 mg/L (95% confidence interval = 10.0 – 25.0 mg/L), and a 1:10 mixture of dispersant/OSE II and

No. 2 fuel oil – 29.4 mg/L (95% confidence interval = 25.0 – 40.0 mg/L).

Table 1. Survival data from toxicity tests

Number Alive

Nominal Concentration (mg/L)	rep.	Dispersant/OSE II 0hr	Dispersant/OSE II 24hr	Dispersant/OSE II 48hr	No. 2 fuel oil 0hr	No. 2 fuel oil 24hr	No. 2 fuel oil 48hr	Oil + Dispersant/OSE II 0hr	Oil + Dispersant/OSE II 24hr	Oil + Dispersant/OSE II 48hr
0 (control)	1	20	20	20	20	20	20	20	20	20
	2	20	20	19	20	20	19	20	20	20
	3	20	20	20	20	20	20	20	20	20
	4	20	20	20	20	20	20	20	20	20
	5	20	20	20	20	20	20	20	20	20
10	1	20	19	17	20	20	17	20	20	19
	2	20	20	17	20	20	19	20	20	18
	3	20	20	20	20	20	12	20	18	18
	4	20	20	19	20	20	9	20	20	17
	5	20	19	18	20	18	10	20	20	16
25	1	20	20	16	20	18	0	20	19	19
	2	20	19	17	20	19	3	20	18	15
	3	20	20	18	20	19	2	20	20	16
	4	20	19	12	20	20	2	20	20	17
	5	20	19	15	20	20	0	20	19	14
40	1	20	19	16	20	20	0	20	19	0
	2	20	20	14	20	19	0	20	20	0
	3	20	20	19	20	20	0	20	20	0

4 20 20 15 20 18 0 20 14 0
5 20 20 17 20 17 0 20 18 2
60 1 20 19 18 20 18 0 20 18 0
2 20 19 16 20 19 0 20 19 0
3 20 19 19 20 16 0 20 19 0
4 20 20 17 20 19 0 20 16 0
5 20 20 16 20 14 1 20 16 1
100 1 20 20 18 20 13 0 20 20 0
2 20 20 18 20 8 0 20 20 0
3 20 19 13 20 9 0 20 20 0
4 20 20 19 20 10 0 20 20 0
5 20 20 16 20 8 0 20 20 0

VII. REFERENCES

Stephen, C.E. 1983. Computer program for calculation of LC50 values. Personal communication.
U.S. EPA. 1984. Revised Standard Dispersant Toxicity Test. Federal Register, Volume 49, Number 139, Wednesday, July 18, 1984, pages 29204 to 29207.

Appendix A. WATER QUALITY DATA FROM TOXICITY TESTS

I. Summary

The acute toxicity of the dispersant – Batch #9820, No. 2 fuel oil, and a 1:10 mixture of dispersant/OSE II and No. 2 fuel oil to *Artemia salina*, is described in this report. The test was conducted for OSEI corp for 48 hours during October 3 to 5, 1990, at the EnviroSystems Division of Resource Analysts, Inc. in Hampton, New Hampshire.

The test was performed under static conditions with five concentrations of each test substance and a dilution water control at a temperature of 20 ± 1◦C. The dilution water was sea water adjusted to a salinity of 20 parts per thousand. Aeration was not employed to maintain dissolved oxygen concentrations above an acceptable level. Nominal concentrations of all three test substances were: 0 mg/L (control), 10 mg/L, 25 mg/L, 40 mg/L, 60 mg/L and 100 mg/L. Nominal concentrations were used for all calculations.

Artemia salina used in the test were 24 hours old at the start of the test and they were all in good condition at the beginning of the study. Exposure of *Artemia salina* to the test substances resulted in the following 48 hours median lethal concentrations (LC50): dispersant/OSE II >100 mg/L, No. 2 fuel oil – 12.6 mg/L (95% confidence interval = 10.0- 25.0 mg/L), and a 1:10 mixture of dispersant/OSE II and No. 2 fuel oil-29.4 mg/L (95% confidence interval = 25.0 – 40.0 mg/L).

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SUMMARY
ENVIRONMENT CANADA'S TOXICITY TEST

Environmental Canada performs Toxicity Testing for determining if a product could gain approval for use in Canada. The level that is considered toxic is 1,000 mg/L or less. A product that exceeds this level is deemed acceptable. The higher the number the less toxic.

Oil Spill Eater II Concentrate, tested at 10,000 mg/L – which shows OSE II Concentrate is virtually non-toxic and far exceeds the level deemed to toxic by Environment Canada.

Rainbow Trout is one of the most sensitive fresh water organisms to test. OSE II proved that even with third party testing by a Foreign Government, OSE II is virtually non-toxic.

By: Steven R. Pedigo
Chairman/OSEI, Corp.121

Environment Canada
Conservation and P ote tion
Emergencies Science Division
River Road Environmental Technology Centre
3439 River Road
Ottawa, Ontario K1A 0H3
May 17, 1993 4808-13-7
Steven R. Pedigo, Chairman,
OSEI Corporation
5545 Harvest Hill
Suite 1116
Dallas, TX 75230
U.S. A.
Dear Mr. Pedigo,

Thank-you for participating in the development of Environment Canada's draft guidelines for assessing the toxicity and effectiveness of oil spill bioremediation agents (OSBAs).

The Tier I toxicity testing is now complete. Our preliminary screening has indicated that the *Daphnia magna* test and the Microtox test were either insensitive or erratic. Therefore, we do not consider these particular tests useful for OSBA evaluation. Comments on the toxicity of your product will thus be limited to those obtained using the 96-hour Rainbow Trout acute lethality test. 'Oil Spill Eater II' had a rainbow trout 96-hour LC50 of greater than 10,000 mg of application solution per litre of water. There was, however, a 23% mean fish mortality at this concentration. Also note that between 24 and 96 hours of exposure to the product, sublethal effects were present. The fish were noted to surface, be on their side, turn dark, exhibit rapid breathing and no swimming. These sublethal effects should be of concern. The effectiveness test analyses are still being performed. You will be notified as soon as those results are available.

If your product meets both the effectiveness and toxicity criteria it will be placed on our Standard List of Oil Spill Bioremediation Agents. Placement on this list is not an indication that the product will be used in the event of an oil spill. The list and test results are public information. They may be provided to oil spill response personnel to enable them to make informed decisions.

Please take note that the placement of a product on our Standard List does not constitute an approval or certification or licensing of your product for use in Canada. Your product may be required to comply with the New Substances Notification Regulations (NSNR) for biotechnology products under the Canadian Environmental Protection Act (CEPA). For information on the draft regulations, please contact the Chief of the New Substances Division at (819) 997-4336 or at the following address: Chief, New Substances Division, CCB, Environmental Canada, P.V.M. 14th Floor, Ottawa, Ontario, K1A 0H3, CANADA.

Sincerely,
Merv Fingas
Chief, Emergencies Science Division

ENVIRONMENT CANADA
TIER I TOXICITY TESTING
FOR EVALUATION OF DRAFT OSBA GUIDELINES

The testing was performed as follows. An application solution of the OSBA was prepared based on instructions provided by the manufacturer/supplier. The highest strength of solution tested was 10,000 mg of application solution per litre of water (approx. a 1:100 dilution). For products in which solids are normally added to the water, suspensions comprised of 10,000 mg of product/combined product per litre of water were prepared for use in the toxicity tests. (If several solids were to be added, they were combined in the appropriate ratio). This initial screening concentration was tested in triplicate. If this concentration was toxic to greater than 50% of the organisms, lower concentrations were tested. Sub-lethal effects on the behavior and/or appearance of the organisms were also made. The toxicity of the product in water was assessed using each of the following three biological test methods, developed and standardized by Environment Canada for these and other applications:

Environment Canada, 1990a. **Biological test method: acute lethality test using rainbow trout.** Environment Canada, Conservation and Protection, Ottawa, Ontario. Report EPS 1/RM/9, 51 pp.

Environment Canada, 1990b. **Biological test method: acute lethality test using *Daphnia* spp.** Environment Canada, Conservation and Protection, Ottawa, Ontario. Report EPS 1/RM/11, 57 pp.

Environment Canada, 1992. **Biological Test method: toxicity test using luminescent bacteria (*Photobacterium phosphoreum*).** Environment Canada, Conservation and Protection, Ottawa, Ontario. Report EPS 1/RM/24, 61 pp.

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TOXICITY TEST SUMMARY USINGCITGO GASOLINE, OIL SPILL EATER II AND FATHEAD MINNOWS

To prove OIL SPILL EATER II rapidly detoxifies hydrocarbons once OSE II is applied, a Toxicity Test was set up with the Physical Engineer of the City of Plano, Texas.

One half gallon of gasoline was poured onto a concrete surface, where ••• gallon of OSE II (pre-diluted 100 to 1 was immediately applied. The treated gasoline was allowed to set for two (2) minutes at which time two (2) gallons of fresh water were used to wash this effluent into a catch basin. Approximately 1 ••• gallons were recovered and sent to Bio-Aquatic Laboratory.

Bio-Aquatic Laboratory performed a Static 48 Definitive Toxicity Test using Fathead Minnows (*Pimphales promeas*). The LC50 was 9,300 mg/L which is a relatively low toxicity level.

This test shows that OSE II when applied to a toxic constituent rapidly reduces toxicity. This detoxifying action of OSE II limits the toxicity of a spill to marine organisms, and will allow Mother Nature's Bacteria to rapidly attack this detoxified spill. The rapid detoxification of a spill shows that OSE II is a beneficial tool for first response cleanup for a spill. This test also shows that if OSE II is used to clean up a parking lot and washed into the storm drain there would be no adverse environmental impact.

By: Steven R. Pedigo
Chairman/OSEI, Corp.124

OSEI CORPORATION
OSE II/GASOLINE/WATER
Toxicity Test Report
DECEMBER 7, 1991
BIO-AQUATIC TESTING, INC.

Prepared by: _____
David Smith,
Aquatic Toxicologist125
BIO-AQUATIC TESTING, INC.
1555 Valwood Parkway, Ste. 100
Carrollton, Texas 75006
Tel: (214) 247-5928
Fax: (214) 241-4474

TOXICITY TEST REPORT – ACUTE

Client . . . . . .OSEI Corporation Laboratory I.D. BO-12-91-2239
Sample . . . . . OSE II/Gasoline/Water Date . . . . . December 7, 1991
Results: The 48-hour LC50 for *Pimephales promelas* exposed to a mixture of OSE II, gasoline, and water was 9,300 mg/L.

SAMPLE COLLECTION

CHEMICAL MEASUREMENTS

TEST PROCEDURES

Pimephales promelas

Approximately one and a half gallons of runoff grab sample from an OSEI Corporation product demonstration was delivered to Bio-Aquatic Testing on December 5, 1991. The sample was manually collected by OSEI personnel. One toxicity test was requested: a static 48-hour definitive toxicity test using the fathead minnow (*Pimephales promelas*).

The sample was analyzed for residual chlorine (EPA Method 330.1, Amperometric Titration Method) and was determined to contain <0.10 mg/L. Sample and laboratory dilution water pH, temperature, conductivity, hardness, alkalinity and D.O. were analyzed and recorded daily.

The 48-hour fathead minnow larval survival test was initiated at 1450 hours, December 6, 1991. Five concentrations were established for testing (200 mg/L, 800 mg/L, 3,000 mg/L, 9,000 mg/L, and 30,000 mg/L) utilizing reconstituted distilled, deionized water as dilution water. The test was set up using distilled water rinsed 500 mL plastic cups as test chambers. Four replicate cups containing five organisms each in 250 mL of test solution were used per dilution. All organisms used were laboratory reared and less than 24 hours old at test initiation. The test was allowed to proceed for 48 hours during which mortality was recorded daily.

A control of four replicate chambers containing five organisms each in 100% synthetic laboratory water was conducted concurrently with the test. There was 100% survival in the control. Data on surviving organisms as well as water quality measurements were recorded on the data sheet. The test ended at 1450 hours, December 8, 1991. The acute toxicity data analysis program provided by the EPA was employed to determine the LC50 values.126

LC50 RESULTS
Pimephales promelas
SUMMARY

LC50 value calculated using the Binomial Method:

CONC. (mg/L)	# EXPOSED	# DEAD	% DEAD	BINOMIAL %
30,000	20	20	100	0.0001
9,000	20	6	30	5.7659
3,000	20	1	5	0.0020
800	20	0	0	0.0001
200	20	0	0	0.0001

The Binomial Test shows that 3,000 and 30,000 can be used as statistically sound conservative 95 percent confidence limits since the actual confidence level associated with these limits is 99.99791 percent.
An approximate LC50 for this set of data is 11,800 mg/L.
LC50 value calculated using the Trimmed Spearman-Karber Method:

Trim	Var. of Ln Est.	LC50	95% Conf. Limits
0.00%	0.17396D-01	9,300 mg/L	7,100 to 12,100 mg/L

The 48-hour LC50 for *Pimephales promela*s exposed to a mixture of OSE II, gasoline, and water was 9,300 mg/L.

BIO-AQUATIC TESTING, INC.

48 – HOUR *PIMEPHALES PROMELAS* ACUTE TOXICITY TEST

CLIENT OSEI Corporation BEGIN DATE 12/06/91
SAMPLE OSE II, Gasoline, Water END DATE 12/08/91
LAB ID **# BO-12-91-2239B** TEST ORGANISM *Pimephales promelas*
DATE COLLECTED 12/05/91 TEST TEMPERATURE ($_{o}$C) 25$_{o}$ ± 1
DATE RECEIVED 12/05/91 PHOTO PERIOD 16 hour light / 8 hour dark
SAMPLE TYPE Grab LIGHT INTENSITY 75 FT-C
TEST TYPE Acute ANALYST W. Smith

EFFLUENT MEASUREMENTS

D.O. @ 30,000 mg/L[1] 8.6/6.6
pH @ 30,000[1] 8.3/8.4
CONDUCTIVITY @ 30,000 (µMHOS) 500
HARDNESS (mg/L as CaCO3) 272.4 ALKALINITY (mg/L as CaCO3) 625.0

DECHLORINATION

RESIDUAL Cl_2 (mg/L) <0.10 ANALYSIS METHOD Amperometric Titration Method (330.1)
DECHLORINATION REAGENT Not Applicable

DILUTION WATER MEASUREMENTS

D.O. @ 100% (mg/L)[1] 8.6/6.9
pH @ 100%1 8.4/8.3
RECEIVING WATER DILUTION WATER Laboratory adjusted
HARDNESS (mg/L as CaCO3) 160.0 ALKALINITY (mg/L as CaCO3) 107.0
[1]Recorded at the beginning and end of each 24-hour exposure period.

SURVIVAL SUMMARY

x % Surv.	x LIVE PER CONC
	100
	100
	100
	95
	70
	0
	% EFFLUENT

CONC												
Control												
200 mg/L												
800 mg/L												
3,000 mg/L												
9,000 mg/L												
30,000 mg/L												
NUMBER LIVE PER REP												
START	24 HOURS				48 HOURS							
a	b	c	d	a	b	c	d	a	b	c	d	
5	5	5	5	5	5	5	5	5	5	5	5	
5	5	5	5	5	5	5	5	5	5	5	5	
5	5	5	5	5	5	5	5	5	5	5	5	
5	5	5	5	5	5	5	5	5	4	5	5	
5	5	5	5	3	3	5	5	3	1	5	5	
5	5	5	5	0	0	0	0	0	0	0	0128	

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EPA in Cooperation with NETAC a Group out of

Pittsburgh University performed Efficacy and ToxicityTesting

on OSE II for the EPA NCP Protocol Development.

The Summary follows

The OSEI Corporation supplied OSE II to Hap Prichard of the US EPA in 1992. The EPA performed two separate tests a 48 hour exposure test and a 96 hour exposure test, on two different species Mysidopsis Bahia, and Menidia beryllina. The Mysidopsis Bahia tests also contained a static renewal LC50 for 48 hours and 96 hours with OSE II, and a 7 day toxicity test as well.

The test information is contained in the five pages following this summary, as well as the freedom of information request that was honored over five (5) years after it was requested for these tests shows the ODSEI Corporation received this information from the US EPA. The test information with the redacted black outs, is as the OSEI Corporation received them, from the US EPA.

Toxicity tests are performed to show the potential effects of a product to marine species. The larger or higher the number the less toxic the product is. LC 50, the LC means lethal concentration, or the concentration of a product to produce death of the test species.

The US EPA's first toxicity test of OSE II was on Mysidopsis Bahia for 48 hours of exposure, and for 96 hours of exposure. The 48 hour exposure toxicity test showed OSE II's toxicity value to be between 5,661 to 7,927 for an average of 6,698. The 96 hour exposure toxicity test showed OSE II's toxicity value to be between 3,125 to 6,250 for an LC 50

of 5,970. These two test shows the US EPA has proven OSE II to be virtually non toxic.

The US EPA static renewal LC 50 with OSE II and the Mysidopsis Bahia was >5,700 for the 48 hour exposure, and >5,700 as well. The EPA established values for OSE II with this species for both exposure times proves OSE II is virtually non toxic.

The US EPA went on to perform a seven (7) day toxicity test with OSE II and the Mysidopsis Bahia. The LC 50 was 2,225 to 3,133, for an LC 50 value of 2,500 which for a seven (7) day toxicity test is phenomenally non toxic.

The US EPA performed toxicity tests on a second species for the EPA/NETAC testing Menidia beryllina. The first test on this species was for an exposure time of 48 hours, and the LC 50 value was 6,250 to 12,500 for an LC 50 value of 8,839. The second test with the Menidia beryllina was for the exposure time of 96 hours, and the value was between 6,250 and 12,500 as well for an LC 50 of 8,839. These two test show the US EPA proving OSE II is virtually non toxic on a second species

These toxicity tests associated with the US EPA/NETAC testing as well as the numerous other toxicity tests that have been performed with OSE II by the US EPA and other governments, and for other governments by the OSEI Corporation overwhelmingly prove OSE II is safe for any marine environments species. These toxicity tests show that when OSE II is utilized for a spill there is real value obtained by using OSE II since it converts a spill to CO 2 and water while limiting and or reducing the toxicity of the spill to the environment.

Steven Pedigo

OSEI Corporation

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL HEALTH AND ENVIRONMENTAL EFFECTS
RESEARCH LABORATORY
RESEARCH TRIANGLE PARK, NC 27711

June 25, 2003

OFFICE OF
RESEARCH AND DEVELOPMENT

Mr. George Lively
Oil Spill Eater International Corp.
13127 Chandler Drive
Dallas, Texas 75243

RECEIVED
BY [illegible] DATE 6-30-03

re: Freedom of Information Act Request HQ-RIN-01971-02

Dear Mr. Lively:

In response to your request for records under the Freedom of Information Act, we were asked to search for and provide data generated using Product C at the Gulf Ecology Division (GED) during the development of oil spill bioremediation protocols. The research involved several laboratories, both within the Office of Research and Development and outside of the Agency.

We are providing these data as an enclosure to this letter, at no cost to you. We also offer a quick explanation of these data in the hopes that it will facilitate your understanding and use .

It is important to note that we used a variety of commercial bioremediation products (CBAs) to develop and evaluate test systems and protocols for the purpose of assessing the efficacy and environmental safety (toxicity) of current and future oil spill bioremediation agents; thus, any data generated with a particular (CBA) was not primarily for the intent of evaluating the product but rather for the purpose of evaluating the test systems under development. These CBAs were provided to us, blind coded, by NETAC–at no time during the collection of these data did we know the actual name of the vendor or product, and thus none of the data will have a vendor's name or product identification associated with it.

In our data, we sometimes refer to Product C as Product 1 - 3 or as CBA C; we have also referred to it by another letter (see manuscript information, below). Data generated at GED was developed through collaborative studies (two cooperative agreements) with the University of West Florida. Throughout the course of evaluating the tests systems, data from more than one CBA might be discussed in notebooks on the same day. Where we have included copies of this data, we have crossed through information that does not respond to FOIA Request HQ RIN-01971-02.

In order to put the data provided in its proper perspective, a copy of a publication and parts of a manuscript are provided to serve as entry points to understanding the data, logs, and materials in this package.

Protocol development utilized a tiered approach of increasingly complex test systems for product evaluation, which is described in more detail in the EPA publication EPA/600/X-93/001 (mentioned below). There were three primary aspects of this research which were conducted at GED that generated data with CBA C:

1. Development of a Tier II Environmental Safety Protocol which focused on the intrinsic toxicity of the bioremediation agent alone and in conjunction with a water soluble oil fraction.

   A manuscript entitled "Evaluation of Protocols to Assess Efficacy and Environmental Safety of Commercial Oil Spill Bioremediation Agents: Agent Toxicity" addresses the Tier II Environmental Safety Protocol; excerpts from this manuscript which include data on Product C are provided. It is important to note that, due to lack of data on all ten products, the products were re-labeled, and **Product C appears in this manuscript as Product "B".** Final editing following review has not been completed, and thus **we request that information in the manuscript not be quoted or cited.** Toxicity data generated at GED that we are providing on this research component includes:

   Menidia beryllina 96-h Static Test with Product C (CBA C)

   Range Finding Acute Test with *Mysidopsis bahia* Using Product C (1 - 3)

   96-h Static Acute Test with 7-day *Mysidopsis bahia* Using Product C (CBA C). [This test was rejected due to low dissolved oxygen concentrations.]

   96-h Static Acute Test with 7-day *Mysidopsis bahia* Using Product C (CBA C)

2. Development of the Tier III Simulated Open Water Test System, which examined the efficacy of a bioremediation agent using a simulated open water/oil slick system.

   The following publication contains a description of Tier III testing as well as summaries of Tier III efficacy data with Product C:

   Lepo, Joe Eugene. 1993. Evaluation of Tier III Bioremediation Agent Screening Protocol for Open Water Using Commercial Agents: Preliminary Report. EPA/600/X-93/001. U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, FL. 27 p.

   Microbiological data supporting this research is identified as:

   Microbiology Associated with Tier III Efficacy Test of Product 1 - 3 (Data from Two Notebooks)

   Analytical Chemistry data for this component is provided as:

   Extraction and Preparation of Samples from Tier III Efficacy Test of Product 1 - 3, Including GC Analysis, and Preparation for GC/MS Analysis

   Gravimetric Data for Tier III Efficacy Test of Product 1 - 3

   Gravimetric/Effluent Data from Tier III Efficacy Test of Product 1 - 3

GC/FID Data for Tier III Efficacy Test of Product 1 - 3

Daily Log of GC/MS Samples

GC/FID Data from Tier III Efficacy Test of Product 1 - 3

GC/MS Data for Tier III Efficacy Test of Product 1 - 3

3. Development of the Tier III Open Water Toxicity Test, which evaluated the toxicity of effluent generated by the Tier III Open Water Test System.

EPA publication EPA/600/X-93/001 (mentioned above) contains summaries of Tier III toxicity data with Product C. Toxicological data supporting this research includes:

7-day Chronic Estimator Test with *Mysidopsis bahia* Using Effluent from Tier III Microcosms with Product C (1 - 3)

We hope you find this explanation, description and records helpful.

Enclosures

Sincerely,

John C. Jones

John C. Jones
Deputy Director for Management
National Health and Environmental
Effects Research Laboratory

TOXICOLOGY

NOTEBOOK: 984

PAGES: 1 - 4

MENIDIA BERYLLINA 96-H STATIC TEST WITH PRODUCT C (CBA C)

Table 3. 48, 96 h, and 7-d LC50 values (95% conf. lim.)[a] for CBAs in static and static-renewal tests using *M. beryllina* and *M. bahia*.

	static LC50		static-renewal LC50		
CBA	48-h	96-h	48-h	96-h	7-d
Mysidopsis bahia					
B	6,698 (5,661-7,927)	5,970 (3,125-6,250)	>5,700	>5,700	2,500 (2,225-3,133)
Menidia beryllina					
B	8,839 (6,250-12,500)	8,839 (6,250-12,500)	---[b]		

[a]Nominal concentrations (mg/L).
[b]Short-term chronic test not conducted.

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Date June 30, 2008

Fresh Water Marine Toxicity Test Summary

South Korea (Minnows)

The OSEI Corporation performed a toxicity test for the Korean Government approval process involving minnows (Pimephales promelas). The toxicity test was a 24 hour acute toxicity test. The LC50 value for this test was 707.11 mg/l at a 20% concentration, which is the concentration the Korean government test required. If you extrapolate the test value, had the test been performed at the OSE II application concentration of 2% instead of 20%, then the LC50 would have been over 1337.11 mg/l which proves OSE II to be virtually non toxic. There are several government agencies around the world that try to force specific tests to be performed at a single concentration without allowing for the application rate of a product. So while they come up with a value at a certain concentration it may, or may not be applicable to every product, which is why we point out the extrapolation calculation for OSE II at the recommended application rate.

Steven Pedigo

Chairman/CEO OSEI Corporation

OIL SPILL EATER II (2%)
ACUTE PRODUCT TEST

June 2008

24-Hour Acute Toxicity Test Results

Pimephales promelas

Prepared for:

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TABLE OF CONTENTS

ACUTE LC50 PRODUCT REPORT

Client . . . . . OSEI, Corporation
Sample . . . . . .Oil Spill Eater II
Project No. OS457
Test Date . . . . . June 2008

Results:

24-hr. *P. Promelas* LC50:	**5,856.34 mg/L**
95% Upper Confidence Limits:	6,265.67 mg/L
95% Lower Confidence Limits:	5,473.76 mg/L

INTRODUCTION

A product identified as Oil Spill Eater II, Concentrate was delivered to Huther and Associates, Inc. on June 26, 2008. One acute toxicity test was conducted: a static acute 24-hour definitive toxicity test using *Pimephales promelas* (fathead minnow). Test procedures followed recommended methods contained in "*Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition*", EPA-821-R-02-012, October 2004.

P. promelas are a freshwater aquatic indicator organism frequently used to evaluate the potential toxicity of a compound or an effluent. The acute toxicity of a compound or effluent is generally measured using a multi-concentration, or definitive test, consisting of a control water and a minimum of five increasing concentrations of product added to control water. The test is designed to provide dose-response information, expressed as the concentration that is lethal to 50% of the test organisms (LC50).

SAMPLE PREPARATION

Oil Spill Eater II was initially prepared for definitive testing by adding the product to distilled, deionized water at a ratio of 50 parts water to 1 part product (2% concentration; stock solution). Seven test concentrations of stock solution were prepared in distilled, deionized water reconstituted to 104 mg/L as $CaCO_3$. The seven concentrations were 250, 500, 1000, 2000, 4000, 8000 and 16,000 mg/L. Dissolved oxygen, pH and conductivity were measured in each concentration prior to test initiation and at 24-hours. The test was conducted at 25^0C in a photoperiod of 16 hours light and 8 hours dark.

TEST DESIGN
Pimephales promelas

The definitive *Pimephales promelas* test was conducted in 300 mL beakers containing 250 mL of test solution. The test was initiated June 28, 2008. Ten *P. promelas* larvae were added to each of two replicate beakers per concentration. Larvae originated from laboratory cultures and were 48-hours old at test initiation. Larvae were fed *Artemia* nauplii prior to test initiation.

A control of two replicate beakers containing ten *P. promelas* larvae each in laboratory water was conducted concurrently with the test. Survival data were statistically analyzed using the Trimmed Spearman-Karber point estimate test to determine the LC50.

RESULTS

Pimephales promelas

The following LC50 value was determined for Oil Spill Eater II (2%):

24-Hour Definitive Test

Conc. (mg/L)	# exposed	# alive	#dead	% survival
Control	20	20	0	100.0
250	20	20	0	100.0
500	20	20	0	100.0
1000	20	20	0	100.0
2000	20	20	0	100.0
4000	20	20	0	100.0
8000	20	1	19	5.0
16000	20	0	20	0.0

Percent Spearman-Karber Trim:	0.00%
Estimated LC50 (mg/L):	**5,856.34**
95% Lower C.L. (mg/L):	5,473.76
95% Upper C.L. (mg/L):	6,265.67

The pH in all solutions was within the organism's tolerance range.

DISCUSSION AND CONCLUSIONS

One LC50 determination was made for Oil Spill Eater II tested at a 2% concentration: 24-hour *Pimephales promelas* LC50: 5,856.34 mg/L. The acute test was conducted from June 28, 2008 to June 29, 2008.

Huther and Associates, Inc.

environmental toxicologists, biologists, consultants

24-HOUR *PIMEPHALES PROMELAS* SURVIVAL

CLIENT: OSE - 28

PROJECT #: 05457

	NUMBER ORGANISMS, 0 HRS		NUMBER ORGANISMS, 24 HRS	
CONC.	A	B	A	B
Con	10	10	10	10
250 mg/L	10	10	10	10
500	10	10	10	10
1000	10	10	10	10
2000	10	10	10	10
4000	10	10	10	10
8000	10	10	10	10
16,000	10	10	10	10
DATE/TIME	mm		mm	
TECHNICIAN	6/28/08 1430		6/29/08 1430	

1156 North Bonnie Brae Denton, Texas 76201 Ph: (940) 387-1026 Fax: (940) 387-1036

OIL SPILL EATER

Test @ 2%

CHEMISTRY MEASUREMENTS

Date	Client	Sample #	ST pH 24hr	ST DO 24hr	Hardness	Alkalinity	Cond. *	Ammonia	Salinity	Analyst
6/28/05										
			ST | 24hr	ST | 24hr				pH@ 96hr	D.O@ 96hr	
	LAB CONTROL		7.84 | 8.60	8.23 | 7.43			390	7.91	7.92	
	250 mg/L		7.86 | 8.59	8.22 | 7.38			389	7.94	8.14	
	500		7.85 | 8.52	8.20 | 7.44			387	7.95	8.27	
	1000		7.85 | 8.48	8.15 | 7.38			387	7.94	8.26	
	2000		7.86 | 8.42	8.10 | 7.53			388	7.93	8.20	
	4000		7.86 | 8.42	8.15 | 7.28			390	7.92	8.21	
	8000		7.85 | 8.31	8.05 | 7.31			393	7.92	8.05	
	16,000		7.81 | 8.14	7.87 | 7.12			399	7.94	8.03	
	LAB WATER CONTROL		7.84		104	64				

TRIMMED SPEARMAN-KARBER METHOD. VERSION 1.5

DATE: JUNE 200 TEST NUMBER: 1 DURATION: 24 H
TOXICANT : OSE II
SPECIES: P. PROMELAS

RAW DATA: Concentration (MG/L)	Number Exposed	Mortalities
.00	20	0
1000.00	20	0
2000.00	20	0
4000.00	20	0
8000.00	20	19
******* 16000.00 BK	20	20

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 5856.34
95% LOWER CONFIDENCE: 5473.76
95% UPPER CONFIDENCE: 6265.67

REFERENCE TOXICANTS

TEXAS COMMISSION ON ENVIRONMENTAL QUALITY

NELAP-Recognized Laboratory Accreditation is hereby awarded to

Huther and Associates, Inc.

1156 Bonnie Brae Street
Denton, TX 76201-2421

in accordance with Texas Water Code Chapter 5, Subchapter R, Title 30 Texas Administrative Code Chapter 25, and the National Environmental Laboratory Accreditation Program.

The laboratory's scope of accreditation includes the fields of accreditation that accompany this certificate. Continued accreditation depends upon successful ongoing participation in the program. The Texas Commission on Environmental Quality urges customers to verify the laboratory's current accreditation status for particular methods and analyses.

Certificate Number: T104704233-08-TX
Effective Date: November 9, 2007
Expiration Date: November 30, 2008

Executive Director
Texas Commission on Environmental Quality

Texas Commission on Environmental Quality

NELAP - Recognized Laboratory Fields of Accreditation

Huther and Associates Inc.
1156 Bonnie Brae
Denton, TX 76201

Certificate T104704233-08-TX
Issue Date: 11/9/2007
Expiration Date: 11/30/2008

These fields of accreditation supercede all previous fields. The Texas Commission on Environmental Quality urges customers to verify the laboratory's current accreditation status for particular methods and analyses.

Matrix: Non-Potable Water

Category / Method: EPA 1000

Analytes:	Code	AA	Analytes:	Code	AA
Pimephales promelas	3410	TX			

Category / Method: EPA 1002

Analytes:	Code	AA	Analytes:	Code	AA
Ceriodaphnia dubia	3315	TX			

Category / Method: EPA 1006

Analytes:	Code	AA	Analytes:	Code	AA
Menidia beryllina	3380	TX			

Category / Method: EPA 1007

Analytes:	Code	AA	Analytes:	Code	AA
Mysidopsis bahia	3395	TX			

Category / Method: EPA 2000.0

Analytes:	Code	AA	Analytes:	Code	AA
Aquatic Toxicity, Acute	10341	TX			

Category / Method: EPA 2002.0

Analytes:	Code	AA	Analytes:	Code	AA
Aquatic Toxicity, Acute	10341	TX			

Category / Method: EPA 2006.0

Analytes:	Code	AA	Analytes:	Code	AA
Aquatic Toxicity, Acute	10341	TX			

Category / Method: EPA 2007.0

Analytes:	Code	AA	Analytes:	Code	AA
Aquatic Toxicity, Acute	10341	TX			

Category / Method: EPA 2021.0

Analytes:	Code	AA	Analytes:	Code	AA
Aquatic Toxicity, Acute	10341	TX			

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Date June 30, 2008

Toxicity Test Summary for a Ceridaphnia Dubia

Water Flea

The OSEI Corporation performed a toxicity test for a land, water, and airborn based species a Ceriodaphnia Dubia (water flea). The estimated LC 50 for this species even at a higher concentration 20%, than OSE II is applied was 2199.62 which shows that OSE II is also virtually non toxic to bugs as well. The extrapolated value for the LC 50 at OSE II normal application rate of 2% would have been over 4000 mg/l, which shows OSE II is virtually non toxic to water fleas.

Steven Pedigo

Chairman/ CEO OSEI Corporation

OIL SPILL EATER II (2%)
ACUTE PRODUCT TEST

June 2008

24-Hour Acute Toxicity Test Results

Ceriodaphnia dubia

Prepared for:

Oil Spill Eater International, Corporation
13127 Chandler Drive
Dallas, Texas 75243
Tel: 972-669-3390

Prepared by: _____
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ACUTE LC50 PRODUCT REPORT

Client . . . . . OSEI, Corporation
Sample . . . . . 2% Oil Spill Eater II
Project No. OS457
Test Date . . . . . June 2008

Results:

24-hr. *C. dubia* LC50: **>16,000.00 mg/L**
95% Upper Confidence Limits: N/A
95% Lower Confidence Limits: N/A

INTRODUCTION

A product identified as Oil Spill Eater II, Concentrate was delivered to Huther and Associates, Inc. on June 26, 2008. One acute toxicity test was conducted: a static acute 24-hour definitive toxicity test using *Ceriodaphnia dubia* (water flea). Test procedures followed recommended methods contained in "*Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition*", EPA-821-R-02-012, October 2004.

C. dubia are a freshwater aquatic indicator organism frequently used to evaluate the potential toxicity of a compound or an effluent. The acute toxicity of a compound or effluent is generally measured using a multi-concentration, or definitive test, consisting of a control water and a minimum of five increasing concentrations of product added to control water. The test is designed to provide dose-response information, expressed as the concentration that is lethal to 50% of the test organisms (LC50).

SAMPLE PREPARATION

Oil Spill Eater II was initially prepared for definitive testing by adding the product to distilled, deionized water at a ratio of 50 parts water to 1 part product (2% concentration; stock solution). Seven test concentrations of stock solution were prepared in distilled, deionized water reconstituted to 104 mg/L as $CaCO_3$. The seven concentrations were 250, 500, 1000, 2000, 4000, 8000 and 16,000 mg/L. Dissolved oxygen, pH and conductivity were measured in each concentration prior to test initiation and at 24-hours. The test was conducted at 25°C in a photoperiod of 16 hours light and 8 hours dark.

TEST DESIGN *Ceriodaphnia dubia*

The definitive *Ceriodaphnia dubia* test was conducted in 25 mL beakers containing 15 mL of test solution. The test was initiated June 28, 2008. Five C. *dubia* neonates were added to each of four replicate beakers per concentration. Neonates originated from laboratory cultures and were 24-hours old at test initiation. Neonates were fed *Selenastrum capricornutum* prior to test initiation.

A control of four replicate beakers containing five *C. dubia* each in laboratory water was conducted concurrently with the test. Survival data were statistically analyzed using the Trimmed Spearman-Karber point estimate test to determine the LC50.

RESULTS
Ceriodaphnia dubia

The following LC50 value was determined for Oil Spill Eater II (2%):

24-Hour Definitive Test

Conc. (mg/L)	# exposed	# alive	#dead	% survival
Control	20	20	0	100.0
250	20	20	0	100.0
500	20	20	0	100.0
1000	20	20	0	100.0
2000	20	20	0	100.0
4000	20	19	1	95.0
8000	20	20	0	100.0
16000	20	17	3	85.0

Percent Spearman-Karber Trim:	0.00%
Estimated LC50 (mg/L):	**>16,000.00**
95% Lower C.L. (mg/L):	N/A
95% Upper C.L. (mg/L):	N/A

The pH in all solutions was within the organism's tolerance range.

DISCUSSION AND CONCLUSIONS

One LC50 determination was made for Oil Spill Eater II tested at a 2% concentration: 24-hour *Ceriodaphnia dubia* LC50: >16,000.00 mg/L. The acute test was conducted from June 28, 2008 to June 29, 2008.

RAW DATA

Huther and Associates, Inc.

environmental toxicologists, biologists, consultants

24-HOUR *CERIODAPHNIA DUBIA* SURVIVAL

CLIENT: OSE 2%

PROJECT #: 05457

	NUMBER ORGANISMS, 0 HRS				NUMBER ORGANISMS, 24 HRS			
CONC.	A	B	C	D	A	B	C	D
CON	5	5	5	5	5	5	5	5
250 mg/L	5	5	5	5	5	5	5	5
500	5	5	5	5	5	5	5	5
1000	5	5	5	5	6	5	5	5
2000	5	5	5	5	5	5	5	5
4000	5	5	5	5	5	5	5	4,
8000	5	5	5	5	5	5	5	5
16,000	5	5	5	5	4,	4,	5	4,
DATE/TIME	6/28/08 1245				6/29/08 1245			
TECHNICIAN	MM				MM			

1445 MacArthur Blvd. Ste. 216 Carrollton, Texas 75007 (972) 242-6844

OIL SPILL EATER

Test @ 2%

CHEMISTRY MEASUREMENTS

Date	Client	Sample #	ST pH 24hr		ST DO 24hr		Hardness	Alkalinity	Cond.	~~Ammonia~~	Salinity	Analyst
6/28/05												
			ST	24hr	ST	24hr				pH @ 96hr	D.O @ 96hr	
	LAB CONTROL		7.84	8.60	8.23	7.43			390	7.91	7.92	
	250 mg/L		7.86	8.59	8.22	7.38			389	7.94	8.14	
	500		7.85	8.52	8.20	7.44			387	7.95	8.27	
	1000		7.85	8.48	8.15	7.38			387	7.94	8.26	
	2000		7.86	8.42	8.10	7.53			388	7.93	8.20	
	4000		7.86	8.42	8.15	7.28			390	7.92	8.21	
	8000		7.85	8.31	8.05	7.31			393	7.92	8.05	
	16,000		7.81	8.14	7.87	7.12			399	7.94	8.03	
	LAB WATER CONTROL		7.84				104	64				

REFERENCE TOXICANTS

Huther and Associates, Inc.

environmental toxicologists, biologists, consultants

ACUTE REFERENCE TOXICANT TEST RESULTS

SPECIES:	*Ceriodaphnia dubia*
CHEMICAL:	Sodium Chloride
DURATION:	48-Hours
TEST NUMBER:	6
TEST DATE:	June 2008
STATISTICAL METHOD:	Spearman-Karber

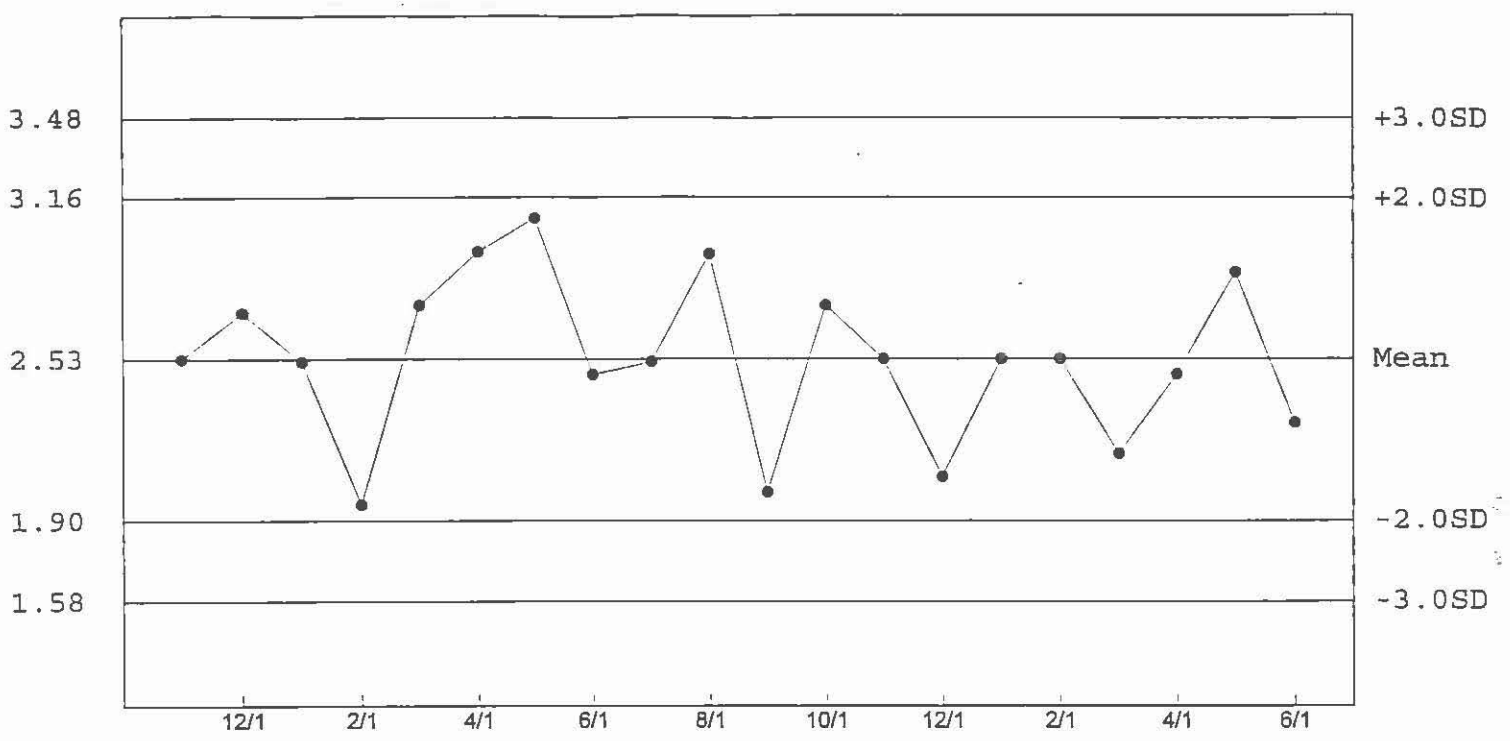
CONCENTRATION (g/L)	NUMBER EXPOSED	NUMBER DEAD
1.0	10	0
1.5	10	0
2.0	10	0
2.5	10	9
3.0	10	10
4.0	10	10

LC50	95% LOWER CONFIDENCE LIMITS	95% UPPER CONFIDENCE LIMITS
2.28 g/L	**2.20** g/L	**2.37** g/L

1156 Bonnie Brae Denton, Texas 76201 (940) 387-1025

Ref. Toxicant Sodium chloride g/L

Ceriodaphnia dubia LC50



n= 20 Mean= 2.53 SD= 0.32 CV= 12.49% Min= 1.96 Max= 3.08

NELAP CERTIFICATE

TEXAS COMMISSION ON ENVIRONMENTAL QUALITY

NELAP-Recognized Laboratory Accreditation is hereby awarded to

Huther and Associates, Inc.

1156 Bonnie Brae Street
Denton, TX 76201-2421

in accordance with Texas Water Code Chapter 5, Subchapter R, Title 30 Texas Administrative Code Chapter 25, and the National Environmental Laboratory Accreditation Program.

The laboratory's scope of accreditation includes the fields of accreditation that accompany this certificate. Continued accreditation depends upon successful ongoing participation in the program. The Texas Commission on Environmental Quality urges customers to verify the laboratory's current accreditation status for particular methods and analyses.

Certificate Number: T104704233-08-TX
Effective Date: November 9, 2007
Expiration Date: November 30, 2008

Executive Director
Texas Commission on Environmental Quality

Texas Commission on Environmental Quality

NELAP - Recognized Laboratory Fields of Accreditation

Huther and Associates Inc.
1156 Bonnie Brae
Denton, TX 76201

Certificate T104704233-08-TX
Issue Date: 11/9/2007
Expiration Date: 11/30/2008

These fields of accreditation supercede all previous fields. The Texas Commission on Environmental Quality urges customers to verify the laboratory's current accreditation status for particular methods and analyses.

Matrix: Non-Potable Water

Category / Method:	Code	AA	Analytes:	Code	AA
EPA 1000					
Pimephales promelas	3410	TX			
EPA 1002					
Ceriodaphnia dubia	3315	TX			
EPA 1006					
Menidia beryllina	3380	TX			
EPA 1007					
Mysidopsis bahia	3395	TX			
EPA 2000.0					
Aquatic Toxicity, Acute	10341	TX			
EPA 2002.0					
Aquatic Toxicity, Acute	10341	TX			
EPA 2006.0					
Aquatic Toxicity, Acute	10341	TX			
EPA 2007.0					
Aquatic Toxicity, Acute	10341	TX			
EPA 2021.0					
Aquatic Toxicity, Acute	10341	TX			